International Journal of Agricultural
Science and Research (IJASR)
ISSN(P): 2250-0057; ISSN(E): 2321-0087
Vol. 7, Issue 5, Oct 2017, 283-294

SOLUBLE SILICA AS A BOON FOR ALLEVIATING TOXIC EFFECTS OF HEAVY METALS ON *VIGNA RADIATA* GROWN HYDROPONICALLY IN SEWAGE

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ABSTRACT

Toxic heavy metals present in sewage induce stress and negatively affect physicochemical and biochemical characteristics of plants. Silicon is beneficial metalloid, for increasing growth of plants especially under adverse conditions. The aim of the study is to evaluate role of soluble silica in reducing toxic effects of heavy metals. In present experimental design Vigna radiata (Mungbean) plants were grown hydroponically for 30-days in sewage and sewage enriched with soluble silica as AgriboosterTM. Effect of silica was investigated, in terms of growth attributes and biochemical characteristics of V.radiata. Results showed that, enrichment of sewage with silica has prevented heavy metal (Lead) accumulation, improved Na+/ K+ ionic balance, and significantly increased (p <0.05) dry weight, carbohydrate content as compared to control. Significant increase (p<0.05) in root length, shoot length and chlorophyll b was observed when sewage was supplemented with silica as compared to sewage. Total chlorophyll and protein content increase was highly significant (p <0.01) on supplementation of sewage with silica as compared to sewage. It was concluded from present study, that soluble silica can reduced toxic effects of heavy metal on V.radiata and improved its quality in terms of sugar and protein content.

KEYWORDS: Agriboostertm, Hydroponics, Protein & Vigna Radiata

Received: Aug 15, 2017; **Accepted**: Sep 04, 2017; **Published**: Sep 23, 2017; **Paper Id**.: IJASROCT201735

Original Article

INTRODUCTION

Sewage water and sludge are used commonly to improve fertility index of soil and productivity (Zeid and Ghate, 2007). Organic matter and essential nutrients present in sewage are beneficial for plants growth (Pathrol *et al*., 2015). However heavy metals like arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) present in sewage are considered as one of the major abiotic stress factor, which inhibit key enzymes of metabolic cycles in plants, damage photosynthetic pigments, increase cell permeability for toxic substances (Dugar and Bafna, 2013; Emamverdian and Ding, 2017). Heavy metals are non biodegradable, accumulate in tissue and cause mutagenic and carcinogenic effect (Nagajyoti *et al*., 2010). Recently, various ex-situ and in-situ physical and chemical methods were adapted to remediate toxic substances of sewage. Out of all these, growing plants hydroponically in sewage enriched with soluble silica has gained a considerable ground so that, plants can utilize mineral nutrients present in sewage without any effects of toxic substance and it could be a better strategy, for improving the crop productivity.

Silicon has not been recognized as an essential metalloid, but it is important for healthy growth and development of plants under stress conditions. Plants uptake silicon below pH 9, mediated by two transporters,

influx (Lsi1), efflux (Lsi2) and tanslocated upwards in shoot via transpiration water flow in xylem. At higher concentration, silicon precipitates as amorphous silica ($SiO_2.nH_2O$) forming phytoliths (Cooke and Leishman, 2011) in cell wall, intercellular spaces of root or shoot tissue of plants. Silicon also form double silica-cuticular layer in leaves (Sangster *et al*., 2001; Lee *et al*., 2001, Ma, 2012). Silicon deposition in cell wall serves as physical and mechanical barrier against external stress (Pozza *et al*., 2015). Silicon alleviates abiotic stress including salinity stress (Lee *et al*., 2010), heavy metal toxicity (Adrees *et al*., 2015; Farooq *et al*., 2016), drought stress (Sapre and Vakharia, 2016), radiation damage, nutrient imbalance, high temperature, freezing (Balakhnina and Borkowska, 2012) and biotic stress like fungi and bacteria (Ma, 2011). Silicon reduces rate of transpiration (Meena *et al*., 2014), increase water use efficiency (Emamverdian and Ding, 2017), and maintain Na^+ and K^+ uptake by plants (Enteshari *et al*., 2011), so that plants can survive under salinity and drought conditions. Silicon reduces heavy metal uptake, by forming Si-metal complex, or by increasing pH of the growth medium (Liang *et al*., 2006). Soluble silica has proven to be more effective in hydroponic systems. In present experimental design, *V.radiata* was grown hydroponically, using sewage and sewage enriched with soluble silica, in order to study whether silica have ability to alleviate toxic effects of heavy metals.

EXPERIMENTAL DESIGN

In present study, seeds of *Vigna radiata* (Mungbeans) variety Samrat 139, of uniform size were surface sterilized with 1% $HgCl_2$. 25 seeds were then placed in each petriplates, lined with Whatman filter paper no. 1 for germination (Kabir *et al*., 2008).

Experimental set up was completely randomized and consist of three different hydroponic cultures, including one control. Three replicates of each treatment were used. Treatment 1) - Modified Hoagland media which served as control, Treatment 2) – Sewage water sample collected from Khan River, near Sanver road industrial area, Indore (M.P), Treatment 3)- Sewage was supplemented with soluble silica in the form of commercial product Agribooster ™ (1litre sewage with 5ml soluble silica). Neutral pH was maintained. Seven days old, fifteen seedlings of *V.radiata* were then transferred from petriplates, to each plastic pots saturated with above mentioned specific treatment solution. Now all sets were cultured hydroponically using static solution culture method, in such a way that it received ample sunlight and air. Plants were harvested after interval of 30-days and were analyzed for their growth attributes and biochemical parameters. Sewage water analysis before and after hydroponic culture was performed. The concentration of heavy metal in *V.radiata* was determined by Atomic absorption spectroscopy.

Growth Attributes

Root and shoot length was recorded by using the standard centimeter scale. For determining fresh weight, three plants were selected at random and for dry weight, plants were dried in a hot air oven over at 80°C for 24 hours. After that, fresh weight and dry weight of the *V.radiata* was recorded using electrical balance (Kabir *et al*., 2008).

Biochemical Analysis

Biochemical changes of *V.radiata* were measured in terms of chlorophyll, carbohydrate and protein content of *V.radiata*.

Chlorophyll Estimation

Chlorophyll content of *V.radiata* was extracted using 80% acetone, and absorbance at 663nm and 645nm were

read in a spectrophotometer (Sadasivam and Manickam, 1992).

Carbohydrate Estimation

Carbohydrate content of *V.radiata* was extracted using 2.5N HCl and estimated by Anthrone method (Hedge *et al.* 1962). Absorption was measured at 630 nm.

Protein Estimation

Protein content of *V.radiata* was determined, according to Lowry *et al.*, (1951) method using Folin-Ciocalteu reagent (FCR). Absorption was measured at 660nm. Amount of protein in the sample was calculated from standard graph and results were expressed in mg/g sample.

Estimation of Heavy Metal Content of *Vigna Radiata*

After 30 days plant samples were dried at 80°C overnight in hot air oven. Heavy metal extract was prepared by dissolving 1gm dry ashes of *V.radiata* in 50 ml Aquaregia. Concentration of heavy metal in extract was determined by using Analytical JENA NOVA 350 AA, based on principle of Atomic absorption spectroscopy (Kumar *et al.*, 2012).

RESULTS AND DISCUSSIONS

The results obtained, out of the experiments conducted in present study, have been shown in figures and tables.

Table 1: Comparison of Growth, Biochemical Parameters and Heavy Metal (Lead) Content of Vigna Radiata Grown in Control (M. Hoagland Media), Sewage and Sewage Enriched with Silica

S. No	Parameters	Control (Hoagland Media)	Sewage Water (A)	Sewage Enriched With Silica (B)	% Change A	% Change B
1	Root Length (cm)	15.00	7.67±0.58 a**	10.00 ±1 a**, b*	-48.89	-33.33
2	Shoot Length (cm)	25.00	17.33±1.53 a*	21.00 ±1 a^{NS}, b*	-30.67	-16.00
3	Fresh Weight (g)	1.33	0.94±0.09 a*	1.10 ±0.10 a^{NS}, b^{NS}	-29.75	-17.50
4	Dry Weight (g)	0.43	0.36±0.04 a^{NS}	0.55 ±0.01 a**, b**	-17.69	27.69
5	Chlorophyll a (mg/g)	0.06	0.057 ±0.006 a^{NS}	0.124 ±0.01 a**, b*	-7.83	101.3
6	Chlorophyll b (mg/g)	0.13	0.07±0.005 a**	0.127 ±0.24 a^{NS}, b*	-47.67	-5.30
7	Total Chlorophyll (mg/g)	0.18	0.14±0.01 a*	0.24 ±0.02 a*, b**	-26.00	33.15
8	Chlorophyll a/b ratio	0.47	0.81 a*	1.01 a*, b^{NS}	73.57	73.57
9	Carbohydrate (% mg)	2.33	3.26±0.416 a^{NS}	3.9 ±0.25 a*, b^{NS}	40.00	67.14
10	Protein(mg/g)	4.37	3.6±0.26 a**	6.0 ±0.50 a**, b**	-17.56	37.40
11	Heavy metal (Pb) (ppm/g)	N.D	0.08	N.D	8	0

^{NS} indicates not significant, * indicates significant, ** indicates highly significant, **N.D**: Not detected.

a-indicates p value as compared to control, **b**- indicates p value as compared to sewage.

Heavy Metal (Lead) Accumulation

In present study, results of Atomic absorption spectroscopy (AAS) showed that toxic heavy metal (lead) was accumulated in *Vigna radiata* grown in sewage while on enrichment of sewage with soluble silica prevented heavy metal accumulation (Table no. 1). Silica may have reduced heavy metal availability in sewage for plants uptake. It is due to co-precipitation of toxic metal-silica complex (Ali *et al.*, 2013), immobilization of toxic metal ion in sewage (Liang *et al.*, 2007), formation of roots exudates (Kidd *et al.*, 2001). Silica also acts as apoplastic barrier in roots, for translocation of toxic metal ions in shoots (Adrees *et al.*, 2015). Bharwana *et al.*, (2016) also showed similar reduction in lead absorption in cotton plants by forming silicates in growth medium.

Table 2: Sewage Water Analysis Before and After Growing Vigna Radiata Plants Hydroponically

S. No	Parameters	Sewage Analysis Before Growing *V.Radiata* Plants	Sewage Analysis After Growing *V.Radiata* Plants	Sewage With Silica Analysis After Growing *V.Radiata* Plants
1	pH	7.70	7.67	7.63
2	Electrical conductivity (dSm^{-1})	4.66	2.14	4.9
3	Calcium (meL^{-1})	14.80	7.90	3.90
4	Magnesium (meL^{-1})	10.60	4.80	12.00
5	Sodium (meL^{-1})	19.00	8.10	18.00
6	Potassium (meL^{-1})	2.60	2.40	0.60
7	Carbonate (meL^{-1})	9.20	2.60	9.10
8	Bicarbonate (meL^{-1})	7.80	4.80	9.80
9	Chloride (meL^{-1})	18.40	8.40	17.40
10	Sulphate (meL^{-1})	12.10	5.20	12.10
11	Sodium Adsorption ratio ($mmol^{L-1}$)	5.33	3.21	5.10

Suitable Ionic Balance (Na^+/K^+)

In plants, suitable ionic balance is important for cell osmoregulation, stomatal function, and activation of many enzymes, protein synthesis, and photosynthesis (Hasanuzzaman *et al.*, 2013). Ionic balance of *V.radiata* was improved in presence of silica, as shown in Table no. (2). It is, because of reduced uptake of Na^+ and increased K^+ uptake of *V.radiata* which is in line with Yin *et al.*, (2013), who also showed that silicon significantly maintained Na^+/ K^+ concentration in shoots under salt stress. It may be due to stimulation of H-ATPase activity, under influence of silica (Liang *et al.*, 2003).

Growth Parameters

Root - Shoot Length and Total Height of *V.Radiata*.

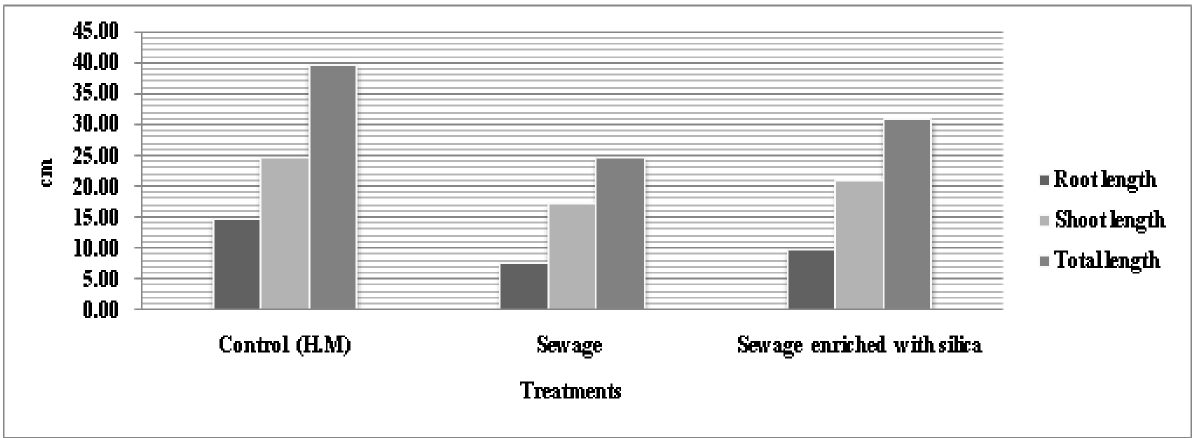

Figure 1: Effect of Soluble Silica on Root - Shoot Length, and Total Height of V.Radiata Grown Hydroponically

As shown in Table 1, reduction in root length was highly significant in sewage as compared to control. This may be because of growing *V. radiata* hydroponically, which leads to direct exposure of roots with harmful components of sewage. Addition of silica in sewage improved root length as compared to sewage (Figure 1). Shoot length and fresh weight of *V.radiata* were significantly reduced in sewage as compared to control. However enrichment of sewage with silica significantly increased shoot length as compared to sewage. Heavy metals present in sewage may have interfered with absorption of essential nutrients from sewage (Lamhamdi *et al.*, 2013; Alia *et al.*, 2015), and diminished plants growth.

Fresh Weight and Dry Weight of *V.Radiata`*

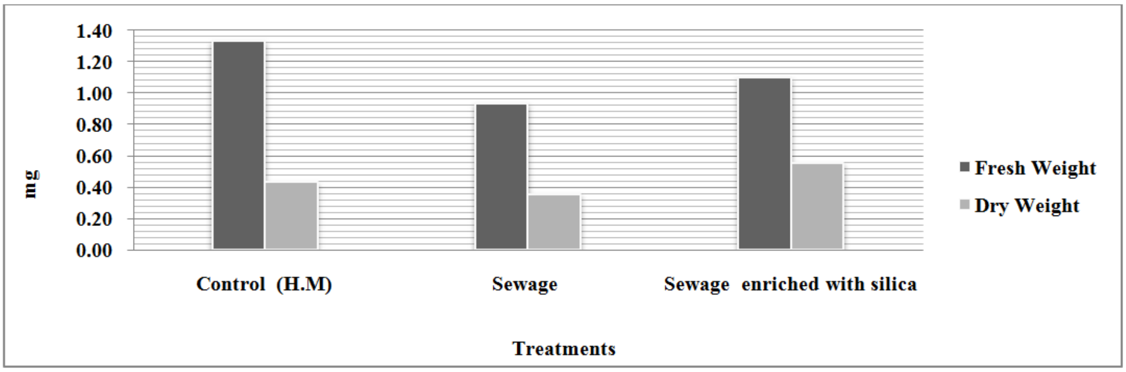

Figure 2: Effect of Soluble Silica on Fresh Weight and Dry Weight of V.Radiata Grown Hydroponically

Dry weight of *V.radiata* was significantly increased by silica, as compared to sewage (Figure 2). This increased dry weight, may be due deposition of silica in solid amorphous form in cell wall, intercellular spaces of root or shoots, tissue of plants and cuticle of leaves (Sangster *et al.*, 2001). Ma, (2012), also reported that, 10% of dry weight of rice was due to silica deposition in biological structure. Lee *et al.*, (2010), observed that, 2.5 mM silicon improved growth and dry weight of hydroponically grown soybean, which was due to regulation of plants hormones like gibberellins and abscissic acid.

Morphological Changes

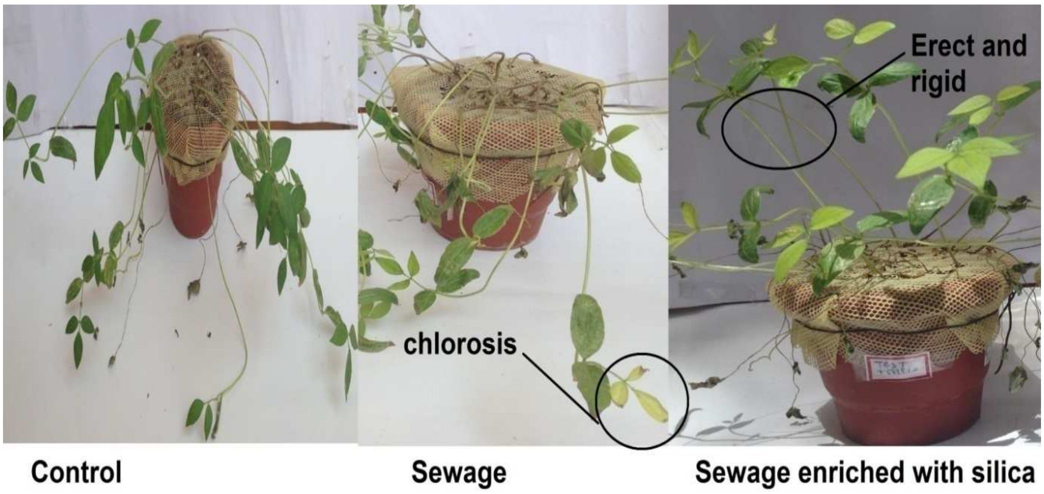

Figure 3: Comparison of Vigna Radiata Plants Grown Hydroponically In Control (Hoagland Media), Sewage and Sewage Enriched With Soluble Silica

As shown in figure 3: Shoots have more upright growth, leaves were more rigid and erect due to prevention of lodging in presence of silica as compared to sewage. In plants, silica deposits in solid amorphous form at biological structure and serve as cementing material to withstand plants against external stress Guerriero *et al.*,2016, Luyckx *et al.*,2017). In present study, sugar content of cells was increased with silica enriched sewage. It may be possible that osmotic potential of cell get reduced and permit more entry of water to maintain turgor pressure which is in accordance with Meena *et al.*, 2014.

BIOCHEMICAL PARAMETERS

Chlorophyll Content

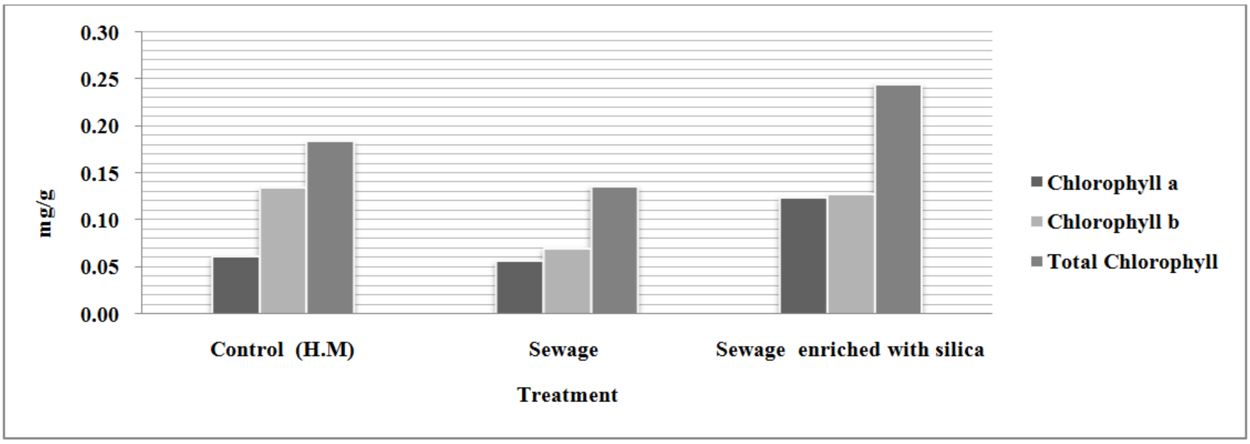

Figure 1: Effect of Soluble Silica on Chlorophyll-A, Chlorophyll-B, and Total Chlorophyll of V.Radiata Grown Hydroponically

As shown in Figure 4, Chlorophyll-b was reduced highly significant with sewage while on enriching sewage with silica has enhanced chlorophyll-b content significantly as compared to sewage. This may be due to oxidation/ reduction of aldehydic group present in structure of chlorophyll-b, caused by free radicals generated under heavy metal stress. Emamverdian and Ding, (2017), showed similar change in chlorophyll-a / chlorophyll-b ratio due to heavy metals stress. Pourrut *et al.*, (2011), revealed that Chlorophyll-b is more sensitive than Chlorophyll-a under lead (Pb) stress. Total chlorophyll content of *V.radiata* was reduced significantly with sewage, while on addition of silica; it was increased significantly, as compared to control. It is possible that, heavy metal (Lead) present in sewage may have inhibited key enzyme delta-aminolevulinic acid dehydratase (ALAD) and reduced uptake of essential nutrients $Mg^{2+,}$ Fe^{2+}, Zn^{2+} (John *et al.*, 2008; Lamhamdi *et al.*, 2013) required for chlorophyll biosynthesis. Heavy metals also aggregate pigment protein complex in photosystems (Sharma and Dubey, 2005), distort chloroplast ultrastructure, and increase chlorophyllase activity (John *et al.*, 2009; Bajguz, 2011), as a consequences chlorophyll content get reduced. Similar decrease in chlorophyll content was also observed in *Shorea robusta,* due to cadmium, arsenic, and lead toxicity (Pant *et al.*, 2014), in *Cajanus cajan* due to arsenic toxicity (Garg and Kashyap, 2017). Silica has increased chlorophyll pigments in *V.radiata,* which is in accordance with Sharifa and Muriefah, (2015), who reported that, on foliar spray with silicon, photosynthetic pigments of Faba beans was significantly increased under heavy metals (Pb and Cd) stress, due to enhanced uptake of essential nutrients like N, P, and K. Malhotra and Kapoor, (2015) also suggested that silica might have stimulated phosphorylation pathway or activation of Mg^{2+} and ATPase activity and enhanced chlorophyll biosynthesis.

Carbohydrate Content

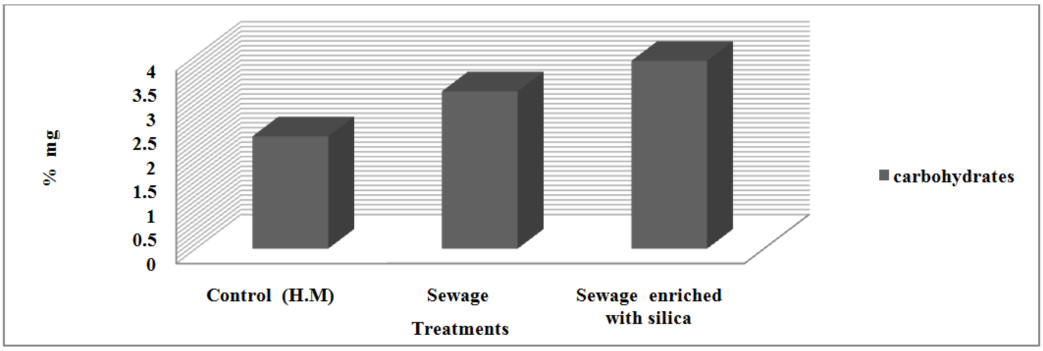

Figure 2: Effect of Soluble Silica on Carbohydrates Content of Vigna Radiata Grown Hydroponically

Carbohydrate content was increased insignificantly in sewage as compared to control, while on addition of silica in sewage, significant enhancement in carbohydrate content was observed as compared to sewage (Figure 5). Toxic heavy metal (lead) present in sewage may have reduced rate of photosynthesis, but plenty of available organic matters and mineral nutrients present in sewage has enhanced carbohydrate biosynthesis which is in accordance with Zeid and Ghate, (2007), who showed similar increase in carbohydrate content of *Phaseolous vulgaris,* due to stimulation of two photosystem by mineral ions present in sewage. Soluble silica alleviates stress conditions, by increasing sugar content and water uptake by plants and reducing osmotic potential of cell. Sharifa and Muriefah (2015) reported increased soluble sugars content in leaves of Faba beans supplemented with silicon. Soluble silica alleviates stress conditions by increasing sugar content and water uptake by plants and reducing osmotic potential of cell. Ding *et al*., (2007) suggested that, silicon has increased soluble sugar contents in leaves of wheat under drought stress which might induce a feedback-regulation of photosynthetic rate.

Protein Content

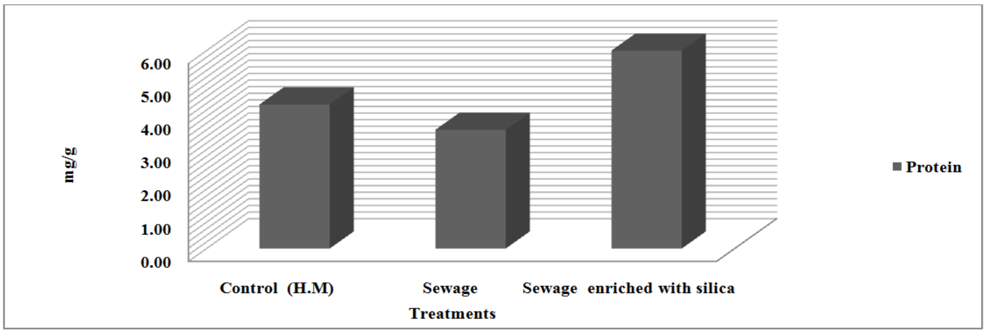

Figure 3: Effect of Soluble Silica on Protein Content of Vigna Radiata Grown Hydroponically

V.radiata is the most important legume due to its high protein content, but its protein content was reduced significantly, when grown in sewage (Figure 6), while, protein content was improved highly, on addition of silica in sewage. Toxic heavy metals present in sewage may have reduced protein content of *V.radiata* which is in accordance with John *et al*., (2008), who showed that, heavy metals like Cd and Pb stimulates protease activity, and cause proteolysis in *L.polyrrhiza*.. Heavy metal (lead), modifies gene expression (Pourrut *et al*., 2011), reduces the protein pool (Singh and Agrawal, 2010), and diminish protein biosynthesis (Bharadwaj *et al*., 2009). It is also possible that, due to presence of heavy metals in sewage, free radical generation may be enhanced. Heavy metal and free radicals may binds with –SH

group of protein and disrupts its tertiary structure and inactivate enzymes involved in protein biosynthesis. This is in line with Gupta *et al*., (2009) who revealed that free radicals generated due to heavy metal stress, cause protein denaturation. While above result is contrary to Senger *et al*., (2008) who reported that, protein content was not much altered by heavy metal (lead) stress. Increased sugar and protein content of *V.radiata,* due to presence of silica is in favor of finding of Malhotra and Kapoor (2015), who showed that, silicon enhance rate of photosynthesis and increase accumulation of solid content in leaf tissues. Silica maintain ionic equilibrium in cell and increase avaibilty of essential nutrients especially nitrogen and phosphorous to plants for protein biosynthesis (Sharifa and Muriefah, 2015). Silica also reduces free radical generation and prevents protein denaturation under heavy metal stress (Bharwana *et al*., 2013). Silica has alleviated salt stress in *Borago officinalis* (Enteshari *et al*., 2011), cadmium stress in rice *(*Farooq *et al*., 2016) and increased protein content significantly. According to Choudhary *et al*., (2007) significant increase in protein content of *S.platensis* may be due to increased proline and antioxidative enzyme, to tolerate heavy metal stress under influence of silica. Presence of soluble silica alleviates the toxic effects of heavy metal, by increasing water retention and uptake of mineral ions, which stimulate enzymes, required for protein biosynthesis, and also helps in damaged cell repairing.

CONCLUSIONS

In present study, toxic components like heavy metals get accumulated in *Vigna radiata* grown hydroponically in sewage and have affected growth, chlorophyll, sugar and protein content. Enrichment of sewage with soluble silica as Agribooster™ has maintained suitable Na^+/K^+ balance and inhibited heavy metals uptake. It can be concluded that addition of silica in sewage has proven better in improving growth and biochemical characteristics as compared to sewage. In parameters like dry weight, total chlolorophyll, carbohydrate and protein content enrichment of sewage with soluble silica has given even better results than modified Hoagland media which is routinely used for growing plants hydroponically. Supplementation of sewage with silica can be a new promising strategy to alleviate toxic effect of heavy metal present in sewage.

ACKNOWLEDGEMENT

We express our sincere thanks to Dr. Rooplekha Vyas, Principal and Prof. A. R. Batham, Head of Department of Biochemistry, Govt. Holkar Science College Indore (M.P.), for providing us necessary laboratory facilities and encouragement.

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